L9: Entry 17 of 26 File: USPT May 27, 1997

DOCUMENT-IDENTIFIER: US 5633234 A
TITLE: Lysosomal targeting of immunogens

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Detailed Description Text (58):
A strategy to utilize enhanced antigen presentation for immunization is to remove antigen presenting cells from the body, culture the cells in vitro, and transfect these cells with an appropriate vector encoding the antigen of interest modified with the LAMP targeting signal, as described above. These transduced antigen presenting cells now express the antigen of interest and can be re-injected into the individual, thereby generating immune responses. An example of this strategy would be the infection or transformation of CD34+ precursors that are differentiating under the influence of GM-CSF into dendritic cells followed by re-injection of these transduced dendritic cells. Utilizing the construct containing antigenic sequences linked to an endosomal/lysosomal targeting signal will enhance the association of peptides derived from a particular antigen with MHC class II molecules on the transduced antigen presenting cells, resulting in significantly more potent systemic T cell dependent immune responses. While the antigen presenting cells transfected in this strategy are preferably autologous cells, any MHC class II.sup.+ cells that effectively present antigen in the host may be used.

Detailed Description Text (67):
In a particularly preferred embodiment, the invention provides a method of treatment for a cancer patient having low tumor burden, such as early in the disease, after resection of a neoplastic tumor, or when the burden of tumor cells is otherwise reduced. In this method, once a tumor-specific cell surface antigen characteristic of the patient's tumor has been identified, a cell population containing autologous stem cells capable of differentiation into antigen presenting cells which will express MHC class II molecules is obtained from the patient. These cells are cultured and transformed by introducing a heterologous or chimeric DNA molecule which encodes a protein containing (1) an N-terminal domain containing at least one epitope of the tumor-specific antigen found on the cells of the patient's tumor, (2) a transmembrane domain and (3) a cytoplasmic domain containing an endosomal/lysosomal targeting signal directing the protein to the lysosomal membrane, i.e., the DNA encodes the immune stimulatory construct described above. The transfected stem cell population is then reintroduced into the patient, where the stem cells differentiate into antigen presenting cells which express MHC class II molecules complexed with T.sub.h epitopes from the tumor-specific antigen. The immune response to the tumor-specific antigen will be enhanced by enhanced stimulation of the helper T cell population.

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L18: Entry 1 of 1 File: USPT May 27, 1997

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US PATENT NO. (1):
5633234

Brief Summary Text (12):
In keeping with the different functions of the cytolytic T cells and helper T cells, the tissue distribution of the MHC molecules that present antigens to these cells is markedly different. The MHC I protein complex that recognizes self or viral antigens is found in virtually all cell types, whereas the MHC II protein that reacts with foreign antigens is found largely in immune cells such as macrophages and macrophage-like cells that either secrete cytokines necessary for T.sub.h cell stimulation of B cells or that require the T.sub.h cell cytokines for their own stimulation. Cells exhibiting MHC II protein are generally called antigen presenting cells.

Brief Summary Text (14):
(1) The MHC class I-related proteolytic system is present in virtually all cells for the purpose of degrading highly abnormal proteins and short-lived molecules or viral proteins. This proteolysis is thought to be non-lysosomal and to involve ATP-dependent covalent conjugation to the polypeptide ubiquitin (Goldberg, et al., Nature, 357:375, 1992). Peptide fragments, possibly in association with a larger proteasome complex, are then postulated to enter into the endoplasmic reticulum or some other type of exocytic compartment (other than the endocytic/lysosomal compartment). There they bind to MHC class I molecules and follow the constitutive secretary pathway from the endoplasmic reticulum through the Golgi to the cell surface where they are presented by the MHC I protein to the CD3-CD8 cytotoxic T cell antigen receptor.

Brief Summary Text (53):
Any strategy which would enhance the presentation of a particular antigen on MHC molecules of host antigen presenting cells would, in fact, enhance the immunization potential of such a viral based strategy for human cancer. The equivalent arguments can be made for generation of enhanced vaccine efficacy for viral infections such as HIV.

Detailed Description Text (23):
Antigens that may serve as the source of preferred antigenic material include tumor antigens, auto-antigens, cell surface proteins found on mammalian cells, proteins of bacteria, protozoa or fungi, including especially proteins found in the cell walls or cell membranes of these organisms, and proteins encoded by the genomes of viruses including retroviruses such as HIV and hepadnaviruses. Particularly preferred antigens are antigens encoded by the genomes of organisms causative for or associated with hepatitis, rabies, malaria, schistosomiasis, cancer, AIDS, yellow fever, dengue fever, equine encephalitis, Rift valley fever, cat scratch fever, viral meningitis. Particularly preferred viral antigens are virally-encoded proteins encoded by the genome of viruses pathogenic to man, horses, cows, pigs, llamas, giraffes, dogs, cats or chickens.

Detailed Description Text (41):
Major efforts in current vaccine research are directed to expression of antigenic proteins by microbial vectors. Recombinant expression vectors may be derived from micro-organisms which readily infect animals, including man, horses, cows, pigs, llamas, giraffes, dogs, cats or chickens. Preferred vectors include those which have already been used as live vaccines, such as vaccinia. These recombinants can be directly inoculated into a host, conferring immunity not only to the microbial vector,

but also to expressed foreign antigens. Preferred vectors contemplated herein as live recombinant vaccines include RNA viruses, adenovirus, herpesviruses, poliovirus, and vaccinia and other pox viruses, as taught in Flexner, Adv. Pharmacol., 21:51, 1990, incorporated herein by reference.

Detailed Description Text (45):
The use of vaccinia as a live virus vaccine in the global campaign to eradicate smallpox made vaccinia an obvious choice for development as a live recombinant vaccine vector. Live recombinant vaccinia viruses expressing close to 100 different foreign proteins have been reported, and a number of these are effective experimental vaccines (reviewed by Moss and Flexner, 1987). Vaccinia is particularly versatile as an expression vector because of its large genomic size, capability of accepting at least 25,000 base pairs of foreign DNA, and its ability to infect most eukaryotic cell types, including insect cells (ibid.). Unlike other DNA viruses, poxviruses replicate exclusively in the cytoplasm of infected cells, reducing the possibility of genetic exchange of recombinant viral DNA with the host chromosome. Recombinant vaccinia vectors have been shown to properly process and express proteins from a variety of sources including man, other mammals, parasites, RNA and DNA viruses, bacteria and bacteriophage. The virus is capable of infecting most mammals, making it a useful vector for studying a broad range of human and animal diseases.

Detailed Description Text (47):
Only about one in a thousand virus particles produced by this procedure is a recombinant. Although recombinant virus plaques can be identified by DNA hybridization, efficient selection procedures have been developed. By using segments of nonessential vaccinia virus thymidine kinase (TK) gene as flanking sequences, the foreign gene recombines into the TK locus and by insertion inactivates the TK gene. Selection of TK virus is achieved by carrying out the virus plaque assay in TK cells in the presents of 5-bromodeoxyuridine. Phosphorylation of the nucleoside analogue and consequent lethal incorporation into viral DNA occurs only in cells infected with TK.sup.+ parental virus. Depending on the efficiency of the transfection and recombination, up to 80 of the plaques are desired recombinants, and the rest are spontaneous TK mutants.

Detailed Description Text (64):
Candidates for cancer immunotherapy would be any patient with a cancer possessing a defined and identified tumor specific antigen whose gene can be cloned and modified by the LAMP lysosomal/endosomal targeting sequences as described herein. Examples include patients with documented Epstein-Barr virus associated lymphomas, patients with HPV associated cervical carcinomas, or patients with a defined re-arrangement or mutation in an oncogene or tumor suppressor gene. It is envisioned that therapy with a vaccine incorporating the tumor antigen linked to the lysosomal/endosomal targeting sequences in a viral vaccine could be utilized at any period during the course of the individual's cancer, once it is identified. It is also possible that in high risk patients, vaccination in order to prevent the subsequent emergence of a cancer with a defined tumor specific antigen could be performed.

Detailed Description Text (66):
In one embodiment, recombinant viral vaccine containing the antigen linked with the lysosomal/endosomal targeting sequence incorporated into a viral vaccine such as vaccinia, would be produced in large quantities as described above and would be injected into the patient at any suitable time during the course of their malignancy. Preferably, the vaccine would be injected at a stage when the tumor burden was low. In an alternative embodiment in which this construct is introduced into the individual's antigen presenting cells, precursors to the antigen presenting cells or mature antigen presenting cells are drawn either from the individual's bone marrow or peripheral blood by vena puncture. These cells are established in culture followed by transduction with the chimeric construct. Once transduction had occurred, these antigen presenting cells are injected back into the patient.

Detailed Description Text (118):
The HA antigen of influenza virus is normally processed and presented in infected cells only in conjunction with the MHC I molecule in the cytotoxic T cell pathway. We have used the influenza virus HA antigen as one model system by which to demonstrate the directed targeting of a viral protein to lysosomes and to the MHC class II/helper T cell pathway.